

# Impact of the Environment on Reproduction from Conception to Parturition

by Frank M. Sullivan

Consideration of environmental influences on human reproduction must include an investigation of the socioeconomic factors that play an important role in embryo-fetal development. Nutritional factors are of prime importance, and modern methods of food production and supply have led to new hazards for pregnant women. For example, pregnant women have been advised in a number of European countries not to eat liver due to the very high concentration (frequently exceeding 100,000 IU per 100 g) of vitamin A. The clear demonstration that periconceptional vitamin supplementation can dramatically reduce the recurrence rate of neural tube defects suggests that folic acid deficiency may be common even in otherwise well-nourished women. Modern methods of food production manufacture and sale have increased the risks of *Salmonella* and *Listeria* infections in pregnancy. The dangers of social drug use, especially alcohol and tobacco, are discussed. The types of adverse effect that can result from chemical exposure during the different stages of pregnancy is reviewed with a discussion of some of the problems of epidemiological studies in attempting to identify toxic chemicals. The extent of the ignorance concerning the reproductive toxicity of industrial and environmental chemicals is emphasized by the fact that data are available on only a small percentage of even the high-production volume (1000 tons/year) chemicals. The evidence of reproductive toxicity from exposure to lead, methyl mercury, polychlorinated biphenyls, polychlorinated dibenzofurans, and hexachlorobenzene are briefly reviewed. Some areas of research needs are discussed with emphasis on nutrition, food production, preparation, and storage and the new problems that may be introduced by genetic manipulation. The lack of information on the reproductive toxic potential of the majority of industrial chemicals necessitates a formal mechanism to screen for possible hazards.

## Environment

Judging from the press and media reports in Europe and the United States, there is a popular conception that many environmental factors have an adverse effect on human reproduction. In recent years there have been many reports that chemical pollution such as dioxin or pesticides or food additives are affecting reproduction and in particular are causing malformations in babies. It is important for those who are involved in reproductive toxicity to examine these claims carefully to see whether any of them are true, and it is also important to look at other environmental influences to see whether there are other more important factors that could affect reproductive health.

One of the definitions of the word "environment" in the Oxford English Dictionary is "the sum total of influences which modify and determine the development of life or character." With this definition of the environment, it is

important that we should not confine our attention merely to chemicals that may affect development but also to other influences as well. If one examines epidemiological studies on any of the adverse outcomes of pregnancy (abortion, malformations, intrauterine growth retardation, stillbirths, or neonatal mortality), the one major factor that is common to practically all of these studies is the influence of the parents' socioeconomic status (SES) (1), that is, what we used to refer to as social class. There is no doubt that the majority of adverse outcomes of pregnancy are highly related to socioeconomic status such that the poorer members of the population have the worst outcomes. The influence of SES is often greater than that of any other individual factor, including genetic background, age, occupation, and parity. If SES plays such an important role in influencing reproduction, it is interesting to try to identify factors involved in such an effect. SES itself cannot account for any adverse effect in pregnancy but must be a surrogate for other factors that are critical in affecting development. An analysis of what these factors might be would suggest that nutrition and differences in social drug use would be two important correlates with SES.

## Nutrition

There is clear evidence that good nutrition is of extreme importance in the production of healthy babies. Not only is

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Department of Pharmacology and Toxicology, United Medical and Dental Schools (St. Thomas Hospital Campus), University of London, London SE17EH, UK.

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it important to have an adequate amount of food, but the quality must also be adequate. An adequate calorie intake is important because weight gain by the mother during pregnancy is an important determinant of birth weight (1). This implies an adequate quantity of food intake by the mother. There is clear evidence that there must also be an adequate supply of trace metals, minerals, and vitamins in the diet to ensure good fetal development. Malnutrition is not a problem that affects only the developing countries but is also a problem affecting the developed Western societies. It is instructive to look at a number of recent issues that have affected certain European countries in recent years and have led to specific warnings for pregnant women.

**Vitamin A.** During 1990, it was found that the vitamin A content of animal liver was extremely high. Studies of liver from different European countries showed ranges of vitamin A content for different types of animal liver, averaging 50,000–100,000 IU/100 g, and values as high as 300,000 IU/100 g were reported for some samples. All types of animal liver were involved—sheep, calf, pig, and chicken. There is evidence that retinoids such as isotretinoin are highly teratogenic and lethal to human embryos. Vitamin A is also known to be teratogenic and lethal to embryos when given in high doses to every animal species in which it has been tested: mouse, rat, rabbit, hamster, guinea pig, pig, and dog. Vitamin A is metabolized in humans to the teratogens 13-*cis*-retinoic acid and all-*trans*-retinoic acid, and there is good reason to believe that excess vitamin A may be teratogenic in humans. From available evidence it would appear that around 20,000 IU/day and above of vitamin A would pose a teratogenic risk to humans, so that people consuming a normal helping of liver (i.e., around 100 g) may be taking in 100–300,000 IU of vitamin A, i.e., about 10 times or more the minimum teratogenic dose. In the face of this evidence, a number of European countries, including the Nordic countries, Great Britain, and Germany, issued warnings that pregnant women should avoid eating liver. The reason for the highest contents of vitamin A in animal liver are not clear yet. There are two sources that have been identified: one is that vitamin A is an important food supplement for farm animals, and vitamin-A-supplemented food is widely used as this encourages growth of the animal. A second use in some countries is that vitamin A injections of 500,000 IU have been given to animals prior to transport to market to improve the quality and color of the meat.

**Folic Acid.** In contrast to the concern about the possible role of excess vitamin A in the diet as a contributory factor to congenital malformations, there is concern about the effects of deficiency of folic acid in the diet. The incidence of neural tube defects, anencephaly, and spina bifida differs from country to country and even within areas within one country, but on average it is around 1 per 300 live births. A woman who has had one child with a neural tube defect has a risk of having a second child affected of about 1:25. Studies originally carried out in the UK and since repeated in a number of other countries have shown that if vitamin supplements are given around the time of conception, the recurrence rate for a neural tube defect can revert

from 1:25 back to the original rate of 1:300. The results of a multinational trial reported recently (2) suggest that the vitamin primarily responsible for this effect is folic acid. The effect of folic acid seems to be independent of social class, so that even well-nourished women may be deficient in folic acid, and it has been proposed that the diet of the UK population should be supplemented with folic acid. One of the concerns about the possible deficiency of folic acid in the diet is that liver has been a traditional important source of folic acid. In a recent survey of the diets of pregnant women in London and Edinburgh (3), it was shown that women living in the UK do not get the recommended daily amount (RDA) of iron and folic acid, whereas the intakes of retinol exceeded the RDA. At issue is whether avoiding liver to prevent excess vitamin A may at the same time lead to a deficit of folic acid.

## Foodborne Infections

Another source of concern relating to animal husbandry, food processing, and storage practice is the risks of infections that have a particular importance for pregnant women. The high prevalence of *Salmonella* infection in poultry and eggs has caused some doctors to advise pregnant women to avoid using uncooked eggs and to take particular care in preparing and cooking poultry. The presence of *Listeria* in soft cheeses and pâtés on supermarket shelves is also of concern for pregnant women. The concern about bovine spongiform encephalopathy (BSE), which can lead to severe neurological damage in farm animals, has raised the question of whether this disease could possibly be transferred to humans, and the possibility that some neurotropic viruses can cross the placenta and affect the developing brain in the fetus is the cause of considerable concern. It is clear from all these examples that problems with nutrition do not merely involve problems of undernutrition in developing countries but also involve malnutrition in developed countries.

## Social Drug Use

There is clear evidence that alcohol consumption, smoking, and excessive caffeine intake may be deleterious during pregnancy (4). The full features of the fetal alcohol syndrome are only seen in women who are chronic alcoholics, but there is evidence that lower amounts of alcohol may also affect fetal brain development. It has been suggested that alcohol may play a role in the etiology of mental retardation similar to Down's syndrome and cytomegalovirus infection. Numerous studies have shown an association between smoking in pregnancy and low birth weight, increased perinatal mortality, and prematurity. There is a strong correlation between alcohol consumption and cigarette smoking, both qualitatively, i.e., women who drink alcohol also tend to smoke cigarettes, and quantitatively, i.e., heavy drinkers also tend to be heavy smokers. There is also a correlation between alcohol, smoking, and caffeine consumption. It is difficult in these situations to apportion blame to the various components of this triad, but all these habits are more common in the lower social classes, and

alcohol and smoking are important determinants of poor pregnancy outcome.

## Unknown Factors

About 2–3% of all babies have recognizable, major congenital malformations at birth. After known causes such as chromosome damage, disease, drugs, and chemicals are accounted for, about 70% of malformations are of unknown etiology. Most researchers believe that the majority of malformations are multifactorial in origin, i.e., there has to be a combination of factors including genetics and environment to result in malformations. The other environmental factors that are involved in the majority of malformations are still unidentified, and this is clearly an important area of future research.

## Adverse Effects from Conception to Parturition

### Pre-implantation Period

From studies in animals and humans, certain types of adverse effects in pregnancy can be clearly defined. These have been discussed at length in a World Health Organization document (5). During the pre-implantation period between conception and implantation, the blastocyst seems to be highly resistant to the action of chemicals, so exposure to toxic substances during this period either results in no adverse effect or death of the blastocyst. It is unlikely that malformations can be induced during this period when the cells of the blastocyst are totipotent so that damaged cells can be replaced by undamaged ones.

### Embryogenesis Period

During the period of embryogenesis, the effects of chemicals may be manifest either as abortions, malformations, or retarded development. Abortion may be the result of a cytotoxic effect on the developing embryo sufficient to result in its death. Abortion may also be a result of fetal malformation. It has been reported (5) that more than 90% of fetuses with malformations are aborted during early pregnancy. It has also been shown that about 60% of first trimester abortions have chromosomal abnormalities such as trisomy, monosomy, or triploidy.

When high doses of chemicals are administered to animals during embryogenesis, three characteristic types of effect have been described (6). The first type of effect is where increasing doses of the chemical results in the appearance of a small percentage of fetuses with malformations, some with retarded development, and an increasing number of fetal deaths. This type of effect is commonly seen with cytotoxic drugs that can produce necrotic damage in the developing embryo, which leads to the production of specific malformations depending primarily on the time of exposure to the chemical. The most sensitive organs are those developing at the time of chemical administration, so that in the early stages of development, central nervous system defects may be produced, and at

later stages limb malformations may be produced. As the doses increase, more extensive tissue damage ultimately leads to death of the embryo.

The second type of effect is where increasing doses of chemical produce an increasing and high proportion of malformed fetuses, some of which may also show evidence of retarded development, but death is not observed until much higher doses are used. This type of effect is due to a specific action, usually on receptors in the fetus. For example, in strains of mice that are sensitive to corticosteroid-induced cleft palate, it has been shown that there is a higher concentration of corticosteroid receptors in the palatal region in the sensitive strains of animals, and this seems to be one factor in the induction of malformations in these mice. Another example is acetazolamide, which inhibits carbonic anhydrase, causing changes in pH in the developing embryo, and which results in the highly specific malformation of right forelimb postaxial reduction defects. As the doses of acetazolamide are increased, other limbs are affected. Another example of this type of effect is the induction of genital malformations induced by sex hormone treatment. All of these can result in a high proportion of malformed fetuses without any increase in embryonic mortality.

The third type of effect that chemicals can exert is where increasing doses lead to an increase in retarded fetuses and subsequent death without the production of specific malformations. Chemicals that produce this type of effect are those which depress, in a nonspecific way, all energy production within the embryos such as, for example, chloramphenicol, which inhibits mitochondrial protein synthesis. This leads to a nonspecific retardation of growth, resulting in retarded development and ultimately in embryonic death, but does not lead to specific malformations.

### Fetogenesis Period

Chemicals acting after the period of embryogenesis, i.e., during fetogenesis, will not normally result in production of gross congenital malformations. During the period of fetogenesis, important developments are general body growth, histological, and functional development of organs, and, most importantly, the histological development of the central nervous system. With regard to the central nervous system, parturition is not a particularly significant event because brain development occurs during the entire period of fetal development and through the first 2 years of postnatal life. Thus, chemicals acting during the later stages of pregnancy (i.e., in the second and third trimesters) might be expected to produce intrauterine growth retardation, inhibition of tissue functional development, and adverse effects on central nervous system development resulting in mental retardation or behavioral deficits.

Animal tests are available for evaluating the adverse effects of chemicals during pregnancy (5). Human epidemiological studies to detect the adverse effects of chemicals on pregnancy present many problems. The overall malformation rate at birth is about 2–3% of babies. For

specific malformations, however, the incidences are much lower, and the common malformations have prevalence rates of around 1:1000. Because chemicals acting as teratogens might be expected to increase the incidence of specific malformations without necessarily altering the overall malformation rate, it is necessary to study a large number of exposed women to detect an increase in malformations. If one uses moderate values for type 1 and type 2 errors, it can be shown that to detect a doubling of a spontaneous malformation with a rate of 1:1,000, about 23,000 women would be necessary to achieve statistical significance, that is, assuming that the background rate was known with some precision (7). Because it is unlikely that so many women would be exposed to one single chemical during pregnancy, small increases in malformation rates are unlikely to be detected by current epidemiological methods. A study of abortion rates is also an indicator of adverse effects in pregnancy. The spontaneous abortion rate among clinically recognized pregnancies is between 10–20%. However, the true abortion rate is much higher than this if one uses sensitive methods such as human chorionic gonadotropin determination of early pregnancy (5).

A sensitive indicator of adverse effects in pregnancy is birth weight (1,8). This has been used with considerable success in the study of social drug influences on pregnancy as well as occupational influences. Reduced birth weight may be measured in two ways that lend themselves to different statistical analytical methods and so complement each other and may lead to the detection of small effects. The first is to measure the mean birth weight of the exposed population compared with the control population and see whether there is any difference. The second method is to measure the incidence of low birth weight babies, i.e., usually defined as those weighing < 2.5 kg, though this obviously would depend on the population being studied. Small differences in mean birth weight may not be of any biological significance, but there is good evidence that babies of low birth weight do not have as good a prognosis as those closer to the mean. Other methods for assessing adverse effects on pregnancy, such as prenatal diagnosis using ultrasound, measurements of  $\alpha$ -fetoprotein or other placental proteins, amniocentesis or chorionic villus sampling, are not suitable for population studies in general.

## Chemical Exposure

People are exposed to different classes of chemicals including therapeutic drugs, socially used and abused drugs, workplace chemicals, chemicals in food and water, and environmental pollutants. Examples of nearly all these classes are known to affect pregnancy outcome and offspring development in humans, though the number of harmful chemicals is actually small. There have been many studies on therapeutic drugs and a good deal of information is available concerning the risks of therapeutic drugs in pregnancy. Because the average life of a drug is relatively limited, much of the information available concerns older drugs, and there is relatively little information on new drugs introduced within the last 5 years. Similarly,

there have been numerous reviews on the effects of social drugs such as alcohol, smoking, and on the abused drugs such as heroin and cocaine.

Chemicals deliberately added to food such as colors and preservatives are normally extensively tested in animals for safety before being used, and it is unlikely that these present any real danger to the population from the viewpoint of human reproduction.

When we move to workplace chemicals and environmental pollutants, however, our ignorance is much greater. Recently the Organisation for Economic Cooperation and Development (OECD) and the European Economic Community (EEC) prepared lists of several thousand chemicals that are produced in amounts of more than 1,000 tons per year, and many of which are produced in amounts of more than 10,000 tons per year. Toxicological data of any type exist for only a few hundred of these chemicals, and reproductive toxicology data exists for probably 100. A small number of these studies have been reviewed (9), and some are currently under review by the EEC, but for the vast majority of industrial chemicals there are no published reproductive toxicology data whatsoever. In the Teratology Information Service, which is run in conjunction with the National Poisons Information Service in the UK, we frequently obtain requests for information on the hazards to pregnant women exposed to chemicals in the workplace, e.g., pottery, dry cleaning, laboratories, chemicals in the home (e.g., paint fumes, dry rot treatments, wood worm treatments, which are frequently carried out while the pregnant woman is living in the house), and pesticide sprays used either industrially in agriculture or in the garden. There is little published information on many of these chemicals, although in the case of pesticides, which have to be registered in most countries, much information on reproductive toxicity must be available, though not in the public arena.

Environmental pollutants known to affect human reproduction include lead (10), methyl mercury (11–13), polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) (14–16), and hexachlorobenzene (HCB) (17). The effects of lead are well known, and most countries have instituted steps to reduce lead contamination of water by replacing lead storage tanks, lead water pipes, and by adding lime to soft water to precipitate lead. Methyl mercury poisoning has occurred in two major ways: one is through the consumption of grain treated with methyl mercury and intended for planting, which has been eaten by large numbers of people during times of famine. This has led to a large number of adult deaths as well as babies born with cerebral palsy, blindness, and severe brain damage. The other cause of methyl mercury poisoning, particularly in Japan, has been consumption of fish contaminated with methyl mercury due to effluent discharged into the sea.

Hexachlorobenzene poisoning has been reported in Turkey, again in the situation where it was used as a fungicide on food grain intended for planting but was consumed by local population. This resulted in severe illness and high mortality in children who were primarily affected by exposure through breast milk.

PCBs have been involved in a number of poisoning incidents, typically in which cooking oil has become contaminated with PCBs due to leaks in heat exchangers during manufacture. These oils also contain PCDFs formed when PCBs are heated to high temperatures. More recently, however, there has been publication of extensive PCB exposure in people living in North America bordering Lake Michigan (18) as a result of eating fish contaminated with PCBs. Studies in nonpregnant adults showed that serum PCB levels correlated with the amount of Lake Michigan fish consumed annually and also with the years of consumption of such fish. Maternal serum and breast milk levels of PCBs similarly correlated with the amount of fish eaten. In the highest fish consumers, it was estimated that for every 0.45 kg of Lake Michigan fish eaten, serum PCB increased by 0.15 ng/mL and transplacental passage of PCBs clearly occurred as umbilical cord levels were similar to those in maternal serum after adjustment for lipid concentration. Because of the levels of PCB in breast milk, infants being fed by the most contaminated women would exceed the recommended upper limit of 1  $\mu\text{g/kg/day}$  for intake of PCBs in adults by a factor of about 25-fold. Dose-related effects were observed in neonates, who described as more jerky, unbalanced, and showing more startle movements and more abnormally weak reflexes the higher the PCB exposure. Effects on development were still present at 4 years of age in the children. At birth there was reduced birth weight, smaller head circumference, and shorter gestation related to PCB exposure in a dose-related way. Although it is difficult in these situations to separate the effects of prenatal exposure from postnatal exposure via breast milk, the authors conclude that a substantial part of the action of the adverse effect of PCBs on child development occurs prenatally due to transplacental transfer (18).

There is evidence that at least part of the prenatal effects of PCB and PCDF exposure *in utero* is mediated by interaction with placental epidermal growth factor (EGF) stimulated receptor autophosphorylation capacity (19). A decrease in EGF receptor phosphorylation was significantly correlated with decrease in birth weight in children affected with oil disease in Taiwan, and there was also a significant correlation between placental EGF receptor and PCB concentrations but not with PCDF concentrations. These results suggest that PCB levels may be more important in determining birth weight than PCDF.

## Research Needs

From the foregoing discussion, it can be seen that there is need for research in a number of different areas relating to environmental effects on pregnancy. There is a need for investigations on the nutritional status of different populations. This refers not only to populations who suffer from undernutrition but also populations exposed in developed countries to food produced by modern methods of farming and food preparation and storage. Well-balanced nutrition is important for normal pregnancy and fetal development, and it is important that the intensive methods of farming that are used currently in developed societies do not intro-

duce distortions in food that could upset the balance of nutrients. Food preparation and storage methods that may increase the risk of infections occurring through food consumption are an increasing problem. It is important that when new methods of pesticide use are developed and genetic manipulation of plants occurs in order to increase disease resistance that other changes are not simultaneously introduced that could affect reproduction.

We know of relatively few chemicals that have adverse effects on reproduction, but we are completely ignorant of the potential reproductive toxicity of more than 90% of industrial chemicals to which people are exposed. There is need for research and resources to be applied to the development of adequate screening methods to try to identify from the many thousands of environmental chemicals those that merit further detailed investigation.

Much information exists in the files of regulatory authorities and the agrochemical industry concerning the reproductive toxicity of currently used pesticides. The majority of this information is not available in the open published literature, and efforts should be made to ensure that this material is made available.

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